의학박사 학위논문

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February 2014

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Dynamic Contrast Enhanced MRI using Macromolecular MR Contrast Agent (P792): Evaluation of Antivascular Drug Effect in Rabbit VX2 Liver Tumor Model

by
Hee Sun Park, M.D.

A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Medicine at Seoul National University College of Medicine

October 2013

Approved by Thesis Committee:

Professor _____Chairman
Professor _____Vice chairman
Professor _____
Professor _____
Professor _____

ABSTRACT

Objectives: To evaluate the utility of dynamic contrast-enhanced MRI using macromolecular contrast agent (P792) for the assessment of vascular disrupting drug effect in rabbit VX2 liver tumor model.

Methods: In 27 VX2 liver tumor-bearing rabbits (14 in the CKD-516 treated group and 13 in the Sorafenib treated group), DCE-MRI was performed at a 3-T scanner before and 4 hours, 24 hours after CKD-516 administration, while 7 days and 14 days after Sorafenib administration, using macromolecular MR contrast agent (P792) (n=7 and n=5) or conventional low molecular MR contrast agent (Gd-DOTA) (n=7 and n=5) in each group. Control group without Sorafenib treatment was added (n=3) in the Sorafenib treatment group. DCE-MR parameters including volume transfer coefficient (K_{trans}) and initial area under the gadolinium concentration-time curve until 60 seconds (iAUC) of tumors were compared between the two different MR contrast agent groups and among time points. DCE-MR parameters were

correlated with histopathology of the tumor using tumor necrosis rate and microvessel density. In addition, reproducibility regarding the measurement of DCE-MRI parameters and source MR image quality was assessed and compared between the two MR contrast agent groups.

Results: In the CKD-516 treated group, subgroup using macromolecular MR contrast agent showed significantly more prominent decrease in K_{trans} and iAUC at 4 hours and 24 hours, compared with that using conventional MR agent. In the Sorafenib treated group, K_{trans} and iAUC more decreased at 7 days and 14 days after treatment in subgroup of macromolecular MR agent than in that of conventional MR agent but without statistical significance. However, changes in DCE MR parameters did not show significant correlation with histologic parameters in both treatment groups. Inter-measurement reproducibility and overall image quality was better, but without statistical significance, in P792 group compared with Gd-DOTA group.

Conclusions: DCE-MRI using macromolecular agent is more appropriate and less gadolinium-toxic in the assessment and

monitoring of antivascular drug effect, than that using conventional MR contrast agent.

Keywords: dynamic contrast-enhanced (DCE) MR, macromolecular MR contrast agent, vascular disrupting agent, CKD-516, Sorafenib, liver cancer, VX2 carcinoma

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VDA = vascular disrupting agent

DCE = dynamic contrast enhanced

K_{trans} = volume transfer coefficient

iAUC = initial area under the gadolinium concentration–time curve until 60 seconds

INTRODUCTION

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is a noninvasive quantitative method of investigating microvascular structure and function. It tracks the pharmacokinetics of injected contrast agent as they pass through the tumor vasculature. It also is a promising technique for in vivo characterization of tumor angiogenesis and evaluation of therapy-induced microvascular changes.

Currently only contrast agent with low molecular weight are used in clinical settings. These products show rapid extravasation through both tumor and normal blood vessels to the extracellular space. On the other hand, contrast agent with high molecular weight shows only limited extravasation through normal blood vessels. The higher permeability of most tumor vasculature allows these contrast agents to penetrate the tumor and thus provide better tumor detection and identification with DCE-MRI. It is generally accepted that low molecular weight gadolinium-based contrast agents often overestimate tumor blood volume and vascular permeability because of their rapid extravasation from blood to the extracellular space in

tumor tissue. Meanwhile, macromolecular contrast agents have shown to provide more accurate tumor characterization and are better able to differentiate tumor vascular permeability.

P792 (Gadomelitol, Vistarem®, Guerbet) is a monogadolinated blood pool agent for MRI. It is 5nm in diameter, which is as five times as large as that of Gd-DOTA (gadoterate meglumine), and 6.5kDa in molecular weight, ten times as large as Gd-DOTA. P792 is a macromolecular Gd-DOTA derivate, originally developed as a blood pool agent with rapid clearance and mainly free renal elimination. As mentioned above, it shows limited diffusion through normal vascular endothelium. According to a previous study comparing DCE-MRI pharmacokinetic parameters using contrast agent of different molecular weights in animal model, relative increase in K_{trans} in tumor tissue compared with normal tissue was highest with the use of macromolecular agent (P792). Permeability parameters are highly dependent on the contrast agent used, and P792 showed most tumor-selective permeability when three (low-, intermediate-, and macro-molecular weight) kinds of contrast agents were compared.

A recent study demonstrated that DCE-MRI is feasible

modality to demonstrate the serial changes of vascular disrupting effect of CKD-516. CKD-516 is an effective vascular disrupting agent (VDA) resulting in rapid vascular shutdown by targeting the tubulin component of tumor vessels and thus leading to tumor necrosis. However, DCE-MRI using macromolecular contrast agent in the assessment of antivascular drug effect has not been explored.

Therefore, the purpose of this study was to validate the utility of DCE-MRI using macromolecular contrast agent (P792) in the assessment of vascular disrupting drug effect of CKD-516 and Sorafenib, which is an another well-known antivascular agent, in rabbit VX2 liver tumor model.

MATERIALS AND METHODS

Animal Model

This study was approved by the Animal Care and Use Committee of Seoul National University Hospital (IACUC No.12-0305). Twenty seven male New Zealand White rabbits weighing 2.5 to 3.5 kg each were used. Before tumor implantation, animals were sedated with an intravenous injection of 5 mg/kg of a 1:1 combination of tiletamine hydrochloride and zolazepam (Zoletil; Virbac, Carros, France) and xylazine hydrochloride (Rompun 2%; Bayer Korea, Seoul, Korea). Through a midline abdominal incision, the left lobe of the liver was exposed and an approximately 5 mm tunnel was made in the subcapsular area of the left lobe of the liver. Afterwards, approximately $1mm^3$ of minced pieces of harvested fresh VX2 carcinoma tissue was locally implanted into the liver through the tunnel. VX2 liver tumors were incubated for 10-12 days after the tumor implantation prior to baseline imaging.

Antivascular Agent Preparation

CKD-516-CKD-516 (Chong Kun Dang Pharm, Seoul, Korea)

was dissolved in 5 mL of saline at a dose of 9 mg/m^2 body surface area, which was equivalent to 0.75 mg/kg.

Sorafenib—For *in vivo* experiments, Sorafenib was dissolved in Cremophor EL/ethanol (50:50; Sigma Cremophor EL, 95% ethyl alcohol) at 4-fold (4X) of the highest dose, foil wrapped, and stored at room temperature. This 4X stock solution was prepared fresh every 3 days. Final dosing solutions were prepared on the day of use by dilution of the stock solution to 1X with water. Lower doses were prepared by dilution of the 1X solution with Cremophor EL/ethanol/water (12.5:12.5: 75).

Experimental Protocol

CKD-516 group – On 12 days after tumor implantation, 14 tumor-carrying rabbits were randomly divided into the P792 subgroup (n=7) and Gd-DOTA subgroup (n=7). Immediately after baseline MR scanning, CKD-516 was administered in 14 animals, by slow intravenous injection over 5 minutes via the auricular vein. For each subject, follow-up MR imaging studies were performed at 4 hours and 24 hours after the baseline MR.

Sorafenib group – After tumor implantation, ten rabbits were randomly divided into the P792 subgroup (n=5) and Gd-DOTA subgroup (n=5). Sorafenib was orally administered to ten rabbits at a dose of 30 mg/kg per day for 14 days. During the Sorafenib administration period, follow up MR imaging studies were done at 7 days and 14 days after tumor implantation. In addition, we set control group (n = 3) in order to obtain the reference information on initial tumor necrosis rate without Sorafenib treatment. Both MR contrast agents were used in the control group (P792; n=2, Gd-DOTA; n=1). A flow chart of the profile based on recommended standards for reporting diagnostic accuracy is presented in Fig. 1.

MR Contrast Agent

P792 (Vistarem; Guerbet) and Gd-DOTA (Dotarem; Guerbet) were used as an MR contrast agent. Their characteristics and three-dimensional structures are given in Fig. 2. P792 has blood pharmacokinetics comparable to that of Gd-DOTA. This new gadolinium chelate is a large-sized compound (6.5 kDa) and diffuses slower than Gd-DOTA. The molecular weight of P792 is almost 10

times that of Gd-DOTA (6.5 kDa vs 0.56 kDa), and its hydrodynamic diameter is about five times larger (5 nm vs 1 nm). This results in a slowing down of the rotational rate of P792, which is known as the "τ_R effect", with respect to that of Gd-DOTA. Therefore, higher relaxivities are obtained with P792 than with Gd-DOTA (Fig.2). The injected dose of P792 was consequently lower than that of Gd-DOTA (50 *u*mol/kg vs 200 *u*mol/kg), so as to obtain similar T1 effects with both contrast media.

MR Image Acquisition

MR imaging examinations were performed using a 3-T MR imaging system (Magnetom Trio, Siemens Medical Solutions, Erlangen, Germany) using a human knee coil. Before each MR scanning, anesthesia was induced as described above. MR examination was performed in the supine position and included the entire liver. After routine localization images, transverse T2-weighted fast spin echo images [repetition time (TR) = 4100 msec, echo time (TE) = 87 msec, slice thickness = 3 mm, matrix = 512 x 358] and T1-weighted images using a gradient echo sequence (TR

= 3.5 msec, TE = 1.5 msec, slice thickness = 3 mm, matrix = 128 x 128) were acquired. For T1 mapping, unenhanced T1-weighted volumetric interpolated breath hold examination (VIBE) images were acquired at each of the three flip angles using following parameters: TR = 3.9 msec, TE=1.4 msec, flip angles (α = 2°, 8° and 15°), slice thickness=3 mm, number of excitation (NEX) = 4, field of view (FOV) = 14 x 14 cm, matrix = 128 x 128, number of slices = 20. Then, DCE-MRI using a free-breathing, radial three dimensional VIBE with k-space-weighted image contrast (KWIC) reconstruction was performed after an intravenous bolus injection of 0.1 mmol/kg of gadoteric acid (Dotarem, Guerbet, Paris, France). The parameters were TR = 3.5 msec, TE = 1.5 msec, flip angle = 11°, slice thickness = 3 mm, NEX = 2, receiver bandwidth = 780 Hz/pixel, FOV = 14 x 14 cm, matrix = 128 x 128, number of slices = 20. The DCE-MRI was continuously scanned 15 times during 180 seconds.

Image Analysis

DCE-MR parametric map acquisition

As most DCE-MR trials of vascular targeting agents, we selected perfusion parameters including voxel-wise perfusion maps of volume transfer coefficient (K_{trans}) and initial area under the gadolinium concentration-time curve until 60 seconds (iAUC) to evaluate the perfusion change induced by CKD-516 and Sorafenib. Using DCE-MR images, parameteric maps of K_{trans} and iAUC were generated using a post-processing software program (Tissue4D, Siemens Medical Solutions, Erlangen, Germany) based on the Tofts model (Fig. 3).

Quantitative measurement of antivascular effect

For the quantitative image analysis, one blinded radiologist with 7 years of experience in MR imaging measured the tumor size. Response to treatment was determined by the change in tumor size defined as the longest diameter measured on axial T2-weighted images. For each subject, percentage change in tumor size between baseline and 24 hour follow-up was calculated using the following equation: Size change (%) = $[(LD_{24hours} - LD_{baseline}) / LD_{baseline}] \times 100$, in which LD is the longest diameter of the tumor.

For each time point, DCE-MR values including K_{trans} and iAUC of the tumor were measured using an operator-defined region of interest (ROI). Each ROI was drawn by outlining the tumor border at the each parametric map of which section included the longest diameter of the tumor. Percentage changes in DCE-MR parameters relative to baseline were calculated as follows: Value change (%) = $[(Value_{given\ time} - Value_{baseline}) / Value_{baseline}] \times 100$.

Reproducibility of MR parameter measurement and qualitative image analysis

For each MR contrast media group, intra-measurement reproducibility was assessed through the calculation of the intraclass correlation coefficient (ICC). Measurement of MR parameter using ROI drawing of the tumor was done three times for the same image set. Agreement was classified as poor (ICC = 0.00-0.20), fair to good (ICC = 0.40-0.75), or excellent (ICC > 0.75). The ICCs were reported with a 95% confidence interval (CI).

For each MR contrast media group, qualitative assessment of source images was performed. All images were qualitatively

assessed by a board-certified abdominal radiologist with 8 years of experience evaluating contrast enhanced MRI. The observer was blinded to the MR contrast agent used. Overall image quality of the dynamic contrast enhanced MRI was scored using a 4-point scale where 1 = poor, 2 = fair, 3 = good, and 4 = excellent.

Histologic Analysis

After 24 hour follow-up MR exam, all rabbits were sacrificed through intravenous injection of 5mL of potassium chloride under deep anesthesia and frozen at -70 ° C in a plastic frame to maintain their posture in order to avoid misregistration between the MR images and the pathologic specimens. Pathologic specimens were sectioned in the transverse plane with a 1 mm interval to match the MR images. For each tumor, a representative microscopic section which would be matched to the corresponding MR image was selected. For each tumor tissue, hematoxylin and eosin (H&E), and CD31 (Dako, Carpinteria, CA, USA) were performed to evaluate the necrotic fraction (NF), and blood microvessel density (MVD) of the tumor, respectively. The proportion of tumor necrosis (necrotic area

to the total tumor surface) was assessed in a semiquantitative manner using the following scoring scale: 1 = 0% to 20%, 2 = 20% to 40%, 3 = 40% to 60%, and 4 = more than 60%. To obtain the histologic vascular parameter of the tumor, hot spots meaning higher vascular density areas than the rest of the tissue were chosen at low magnification (x 40) and CD31 stained vessels were counted at high magnification (x 200, 0.544 mm^2). The mean of three measurements in the hot spots was used as the mean MVD of the tumor.

Statistical Analysis

In order to determine whether there were differences in interval changes of the tumor size between each subgroup of the treated groups, the Mann-Whitney test (CKD-516 treatment group) or Kruskal-Wallis test (Sorafenib treatment group) was used. For the animals that survived until the 24 hours follow-up in the CKD-516 treated group and 14 days follow up in the Sorafenib treated group, serial change in DCE-MR parameters at different time points were evaluated and compared between the P792 subgroup and Gd-DOTA subgroup using Mann-Whitney test. Spearman rank

correlation test was performed to assess the correlation between MR parameters at 24 hour- (CKD-516 group) or 14 days- (Sorafenib group) follow-up and corresponding histologic features such as NF and MVD. Intra-measurement reproducibility of the DCE-MRI was assessed through the calculation of the intraclass correlation coefficient (ICC). Comparison of the reproducibility of MR parameter measurement between the two MR contrast agents was done using Z-test (comparison of ICC). Overall image quality of the two MR contrast agent groups was compared using Mann-Whitney test. A P value of less than 0.05 was regarded to be of statistical significance. All statistical analyses were performed using MedCalc software version 12.2.1.0 (MedCalc Software, Mariakerke, Belgium).

RESULTS

In the CKD-516 group, one rabbit out of 14 rabbits died in the Gd-DOTA subgroup after 4hr MR scanning. In Sorafenib group, one rabbit in the P792 subgroup and two rabbits in the Gd-DOTA subgroup died during the 14-day Sorafenib administration period. Finally, 13 animals in the CKD-516 group (n = 7 in the P792 subgroup and n = 6 in the Gd-DOTA subgroup), 7 animals in the Sorafenib group (n = 4 in the P792 subgroup and n = 3 in the Gd-DOTA subgroup), and 3 animals in the Sorafenib control group were included in the DCE MR and histologic analysis.

Part I. CKD-516 Treatment

Comparison of tumor size change between each subgroup after CKD-516 treatment

In the P792 subgroup, mean diameter of the tumors was 0.81 ± 0.14 cm (mean±standard deviation) before treatment and 0.73 ± 0.14 cm at 24 hour after treatment. Tumor size change was -9.96 ± 7.53 %. In the Gd-DOTA subgroup, it was 0.86 ± 0.11 cm before treatment, 0.78 ± 0.08 cm at 24 hour after treatment, and

the size change was −8.8 ± 5.64 %. Tumor size reduction was not statistically significant in both subgroups (P > 0.05) at 24 hour after treatment, and the degree of tumor size change was not significantly different between the P792 subgroup and Gd-DOTA subgroup (P > 0.05).

Comparison of DCE-MR parameters between the P792 and Gd-DOTA subgroup after CKD-516 treatment

The median values of the percentage change of DCE-MR parameters relative to baseline for each group at each time point are summarized in Table 1 and Figure 4(a)-(d). Overall relative changes in K_{trans} and iAUC percentage, 4 hours and 24 hours follow up after CKD-516 treatment was greater in the P792 contrast media subgroup compared with those of Gd-DOTA subgroup. The difference was statistically significant except ΔiAUC 4 hours follow up after CKD-516 treatment.

Correlation of DCE-MR parameters with histolopathology in the P792 and Gd-DOTA subgroup after CKD-516 treatment

NF was 1.857 ± 0.69 in the P792 subgroup and 2 ± 0.632 in the Gd-DOTA subgroup. MVD was 30.8 ± 8.6 in the P792 subgroup and 34.2 ± 11.7 in the Gd-DOTA subgroup. In the 13 rabbits (n=7 in the P792 subgroup and n=6 in the Gd-DOTA subgroup), DCE-MR parameters (K_{trans} and iAUC) at 24 hour follow-up exams and corresponding histologic features including NF and MVD were correlated. However, neither NF nor MVD revealed significant correlation with DCE-MR parameters ($P > 0.05$) (Table 2).

Part II. Sorafenib Treatment

Comparison of tumor size change and histopathology between control and treated subgroup after Sorafenib treatment

Because DCE-MR was done only 7 days and 14 days after Sorafenib treatment, initial tumor size measurement was not available. Instead, we compared the tumor sizes of control group (n = 3, without Sorafenib treatment) and Sorafenib treated group (n = 7) 14 days after treatment. In the control group, mean diameter of

the tumors was 1.53 ± 0.65 cm (mean ± standard deviation). Mean diameter of the Sorafenib treated group was 1.42 ± 0.41 cm. There was no significant difference in the tumor sizes between the control group and the Sorafenib treated group ($P > 0.05$). NF was 2 ± 1.414 in the P792 subgroup, 1.667 ± 0.577 in the Gd-DOTA subgroup, and 1.333 ± 0.577 in the control group. Average NF of treated subgroups was higher than that of control group but without statistical significance. MVD was 28.8 ± 10.4 in the P792 subgroup, 34.2 ± 11.7 in the Gd-DOTA subgroup, and 39.0 ± 9.4 in the control group. MVD was not significantly lower in the Sorafenib treated group than control group ($P > 0.05$).

Comparison of DCE-MR parameters between the P792 and Gd-DOTA subgroup after Sorafenib treatment

The bar graph showing percentage change of DCE-MR parameters at 14 days after Sorafenib treatment relative to 7 days after treatment for each group is demonstrated in Figure 6. Overall relative changes in K_{trans} and iAUC percentage, 14 days follow up after Sorafenib treatment was higher in the P792 contrast media

subgroup compared with those of Gd-DOTA subgroup. However, the difference did not reach the statistical significance.

Correlation of DCE-MR parameters with histopathology in the P792 and Gd-DOTA subgroup after Sorafenib treatment

In the 10 rabbits (n=4 in the P792 subgroup, n=3 in the Gd-DOTA subgroup, and n=3 in the control group), DCE-MR parameters (K_{trans} and iAUC) at 14 days follow-up image and corresponding histologic features including NF and MVD were correlated. Neither NF nor MVD revealed significant correlation with DCE-MR parameters ($P>0.05$).

Part III. Reproducibility and Image Quality

Reproducibility of MR parameter measurement in the two MR contrast agent groups

There were 33 MR image sets in total in the P792 group (n=21 in CKD-516 treatment group, n=8 in Sorafenib treatment group, and n=4 in the Sorafenib control group), and 26 MR image

sets in the Gd-DOTA group (n=18 in CKD-516 treatment group, n=6 in the Sorafenib treatment group, and n=2 in the Sorafenib control group). Result of ICCs is demonstrated in the Table 5. In the P792 group, K_{trans} and iAUC showed ICC values of 0.95 (95% CI, 0.93-0.98) and 0.93 (95% CI, 0.90-0.96), respectively. In the Gd-DOTA group, K_{trans} and iAUC showed ICC values of 0.92 (95% CI, 0.89-0.94) and 0.89 (95% CI, 0.85-0.91), respectively. Intra-measurement agreement was higher in P792 group than in Gd-DOTA group without reaching statistical significance ($P > 0.05$).

Qualitative assessment of overall image quality

From the qualitative analysis based on a ranking of 1 to 4, visual scores were higher in the P792 group (3.52 ± 0.63, mean ± standard deviation) than the Gd-DOTA group (3.19 ± 0.71) (P= 0.12).

Table 1. Relative changes in DCE-MR parameters at 24-hour follow-up studies of the two contrast media subgroups in the CKD-516 treated group.

DCE-MR parameters	P792 (n = 7)	Gd-DOTA (n = 6)	P value*
Δ K_{trans} (%)			
4 hours	−117.68 (−215.63, 10.00)	−47.33 (−81.36, −25.86)	0.045
24 hours	−206.56 (−456.52, −16.13)	−76.37 (−150.00, −27.38)	0.025
Δ iAUC (%)			
4 hours	−82.83 (−258.11, 23.44)	−48.22 (−51.25, 81.69)	0.097
24 hours	−397.99 (−815.82, 9.02)	−42.69 (−163.28, 20.96)	0.028

Note. - Data are mean values and the data in parentheses are ranges. * Mann-Whitney test.

Table 2. Correlation of DCE-MR parameters at 24-hour follow-up studies of the two contrast media subgroups with histologic parameters in the CKD-516 treated group.

DCE-MR parameters	NF (n = 13)		MVD (n = 13)	
	Correlation coefficien	P value*	Correlation coefficient	P value*
K_{trans} (min^{-1})	-0.21	0.35	0.26	0.29
iAUC (mmol/sec)	-0.25	0.28	0.21	0.41

Note. - *Spearman's correlation.

Table 3. Relative changes in DCE-MR parameters at 14-day follow-up studies of the two contrast media subgroups in the Sorafenib treated group.

DCE MR parameters	P792 (n = 4)	Gd-DOTA (n = 3)	control (n = 3)	P value*
ΔK_{trans} (%)				
14 days	−66.77 (−96.14, −44.23)	−52.3 (−71.37, −32.11)	−31.13 (−46.23, −18.93)	0.177
ΔiAUC (%)				
14 days	−55.7 (−66.12, −38.83)	−55.38 (−77-61, −30.02)	−18.59 (−33.13, 0.12)	0.081

Note. - Data are mean values and the data in parentheses are ranges. *Kruskal-Wallis test.

Table 4. Correlation of DCE-MR parameters at 24-hour follow-up studies of the two contrast media subgroups with histologic parameters in the Sorafenib treated group.

DCE-MR parameters	NF (n = 10)		MVD (n = 10)	
	Correlation coefficient	P value*	Correlation coefficient	P value*
K_{trans} (min^{-1})	-0.14	0.51	0.19	0.27
iAUC (mmol/sec)	-0.29	0.34	0.15	0.26

Note. - *Spearman's correlation.

Table 5. Intra-measurement agreement of DCE-MR parameters in the two MR contrast agent groups.

	P792 group (n = 33)	Gd-DOTA group (n = 26)	Z statistics	P value*
ICC				
K_{trans}	0.95 (0.93, 0.98)	0.92 (0.89, 0.94)	-1.63	0.35
iAUC	0.93 (0.90, 0.96)	0.89 (0.85, 0.91)	-2.17	0.28

Note. - Data are intraclass coeffieicnts (ICCs) and the data in parentheses are 95% confidence interval. *Z- test.

Figure 1. A flow chart of the profile based on recommended standards for reporting diagnostic accuracy. DCE-MRI = dynamic contrast-enhanced MRI, NF = tumor necrosis fraction, MVD = microvessel density.

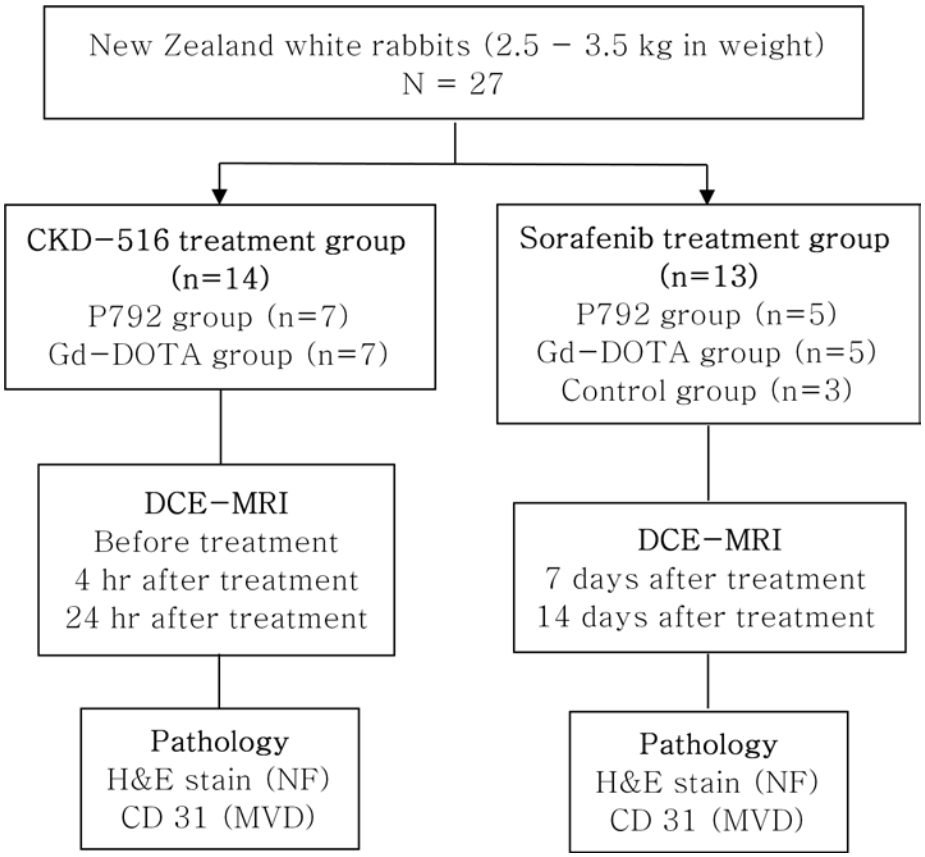
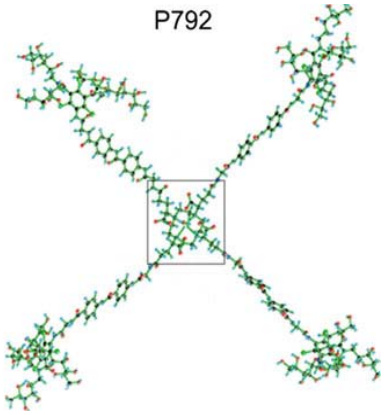


Figure 2. List of contrast agent properties.

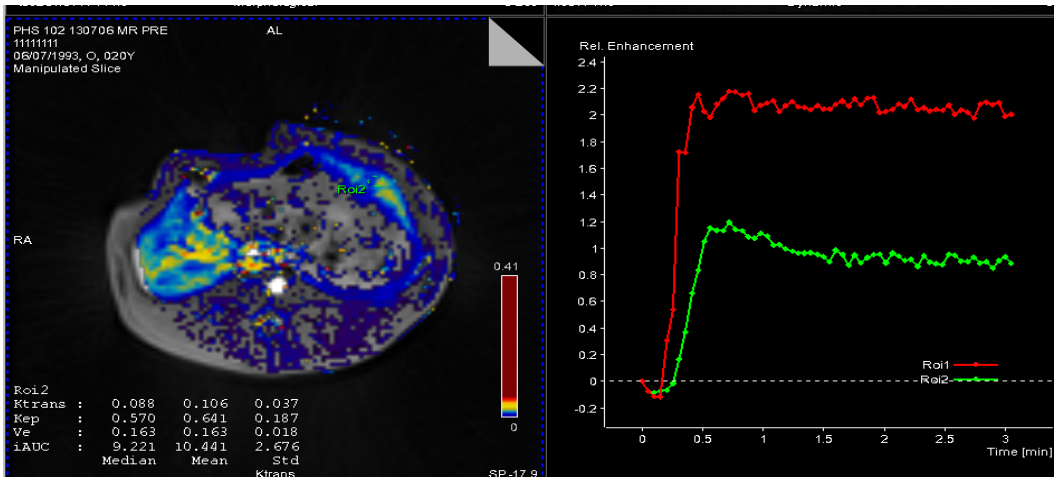
	P792	Gd-DOTA
Molecular weight (kDa)	6.5	0.56
Hydrodynamic size (nm)	5	1
Gd (atom/mol)	1	1
3D molecular modeling		
Relaxivities		
r1 ($s^{-1}.mM^{-1}$)	12	3.3
r2 ($s^{-1}.mM^{-1}$)	68	4.1
Dose (umol/kg)	50	200
Status	preclinical investigation	clinical approval

Note. - Diagrams inserted in the figure are adopted from the reference; Delrue LJ, Casneuf V, Van Damme N, et al. Assessment

of neovascular permeability in a pancreatic tumor model using dynamic contrast-enhanced (DCE) MRI with contrast agents of different molecular weights. Magma 2011; 24:225-232.

Figure 3. Changes of DCE-MR parameters using P792 after CKD-516 treatment. K_{trans} maps show a marked reduction of K_{trans} of the tumor: mean of 0.106 min^{-1} at baseline (a) to 0.065 min^{-1} at 24 hour follow-up (b). Time-intensity curve demonstrates a rapid increase of signal intensity within the tumor at baseline study however, at 24 hour follow-up study, gradual increase of the signal intensity with lower peak intensity (red line: aorta, green line: tumor) resulting in lower K_{trans} value.

(a)



(b)

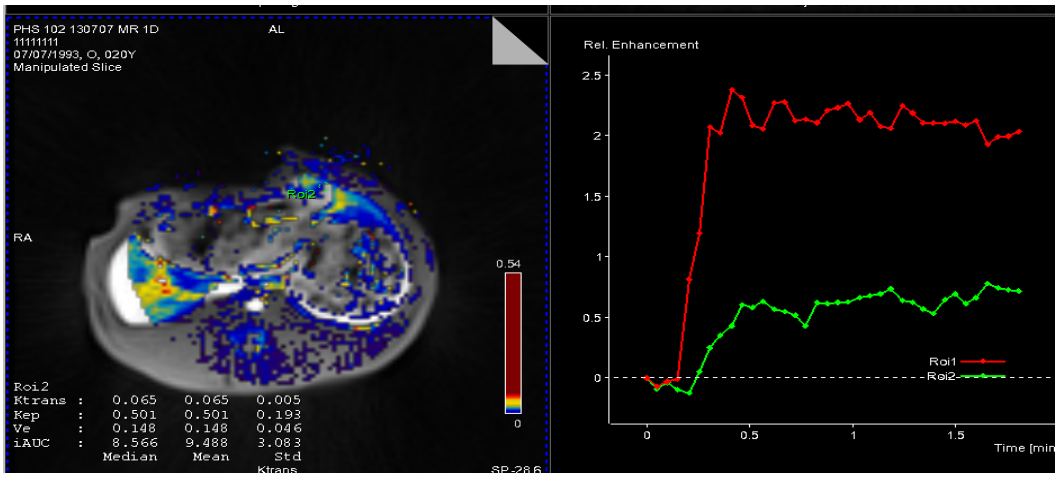
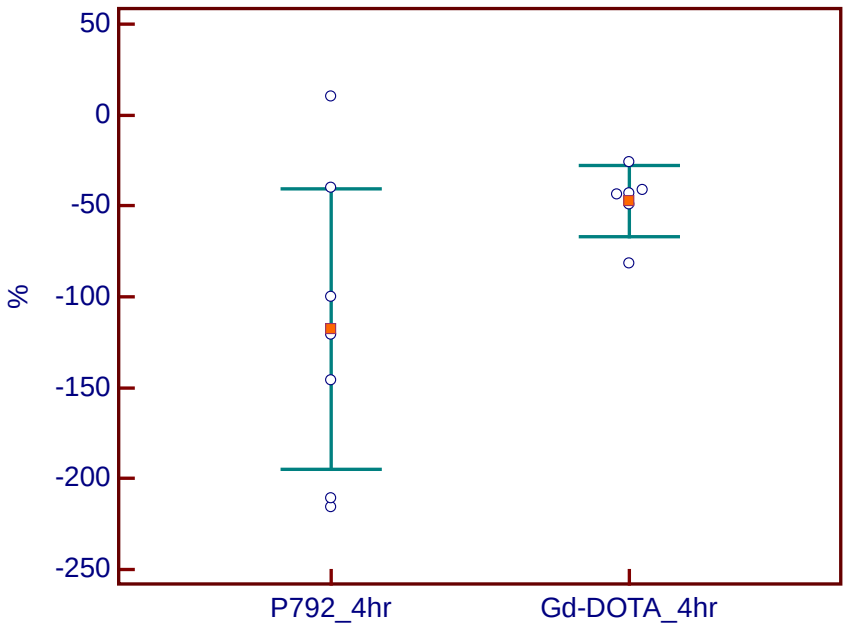
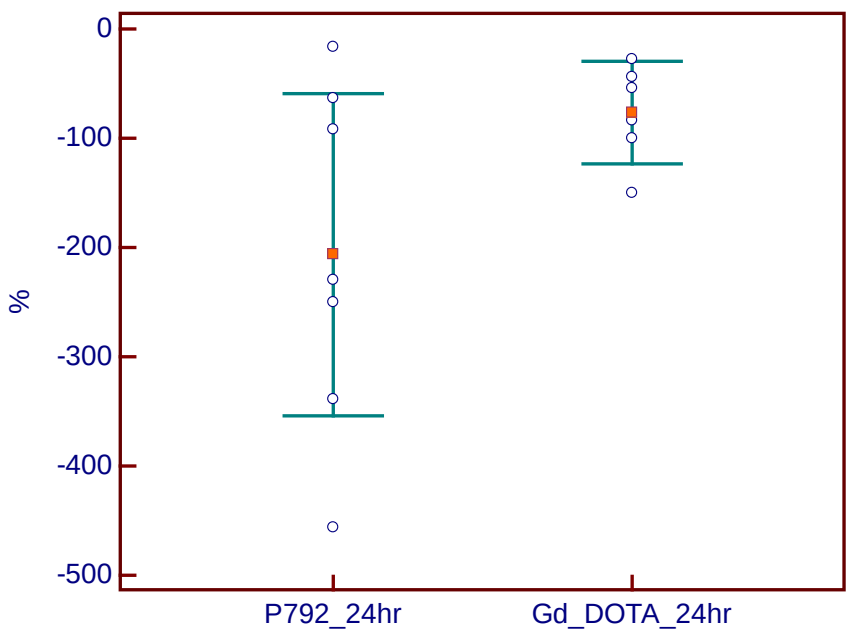

PHS 102 130707 MR 1D
11111111
07/07/1993, O, 020Y
Manipulated Slice
AL
RA
Roi2
0.54
0
Roi2
Ktrans : 0.065 0.065 0.005
Kep : 0.501 0.501 0.193
Ve : 0.148 0.148 0.046
iAUC : 8.566 9.488 3.083
Median Mean Std
Ktrans
SP:28.6
Rel. Enhancement
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
Time [min
Roi1
Roi2

Figure 4. Bar graphs showing relative changes in DCE-MR parameters at follow up studies of the subgroups after CKD-516 treatment. (a)Δ K_{trans} (%) at 4 hours, (b)Δ K_{trans} (%) at 24 hours, (c)Δ iAUC (%) at 4 hours, and (d)Δ iAUC (%) at 24 hours after CKD-516 treatment. Error bars indicate 95% CI for mean values.

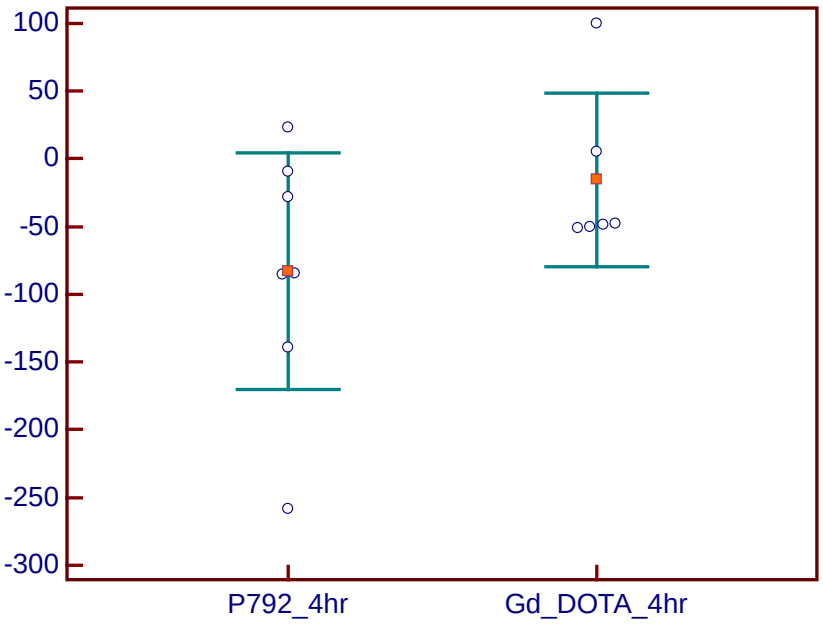
(a)



(b)


0
-100
-200
-300
-400
-500
%
P792_24hr
Gd_DOTA_24hr

(c)


100
50
0
-50
-100
-150
-200
-250
-300
P792_4hr
Gd_DOTA_4hr

(d)

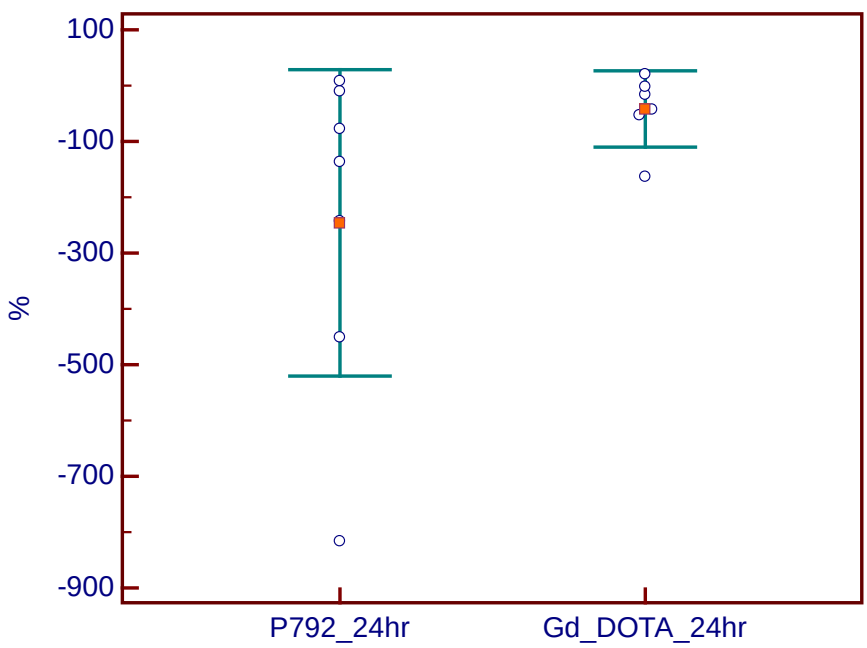
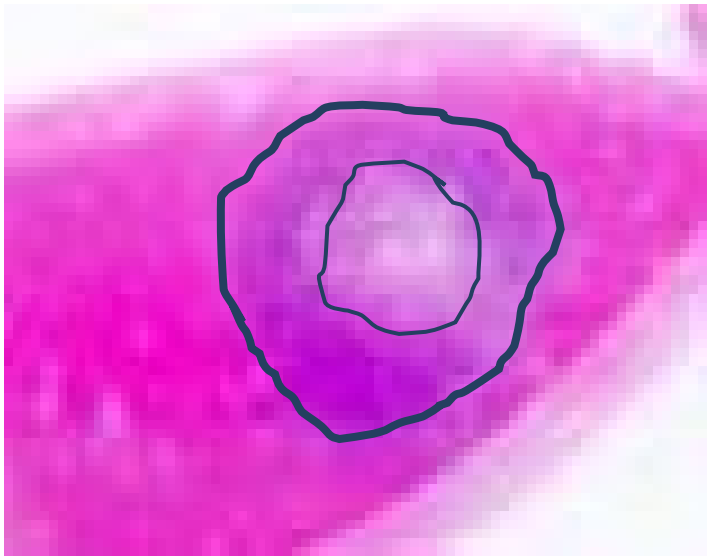
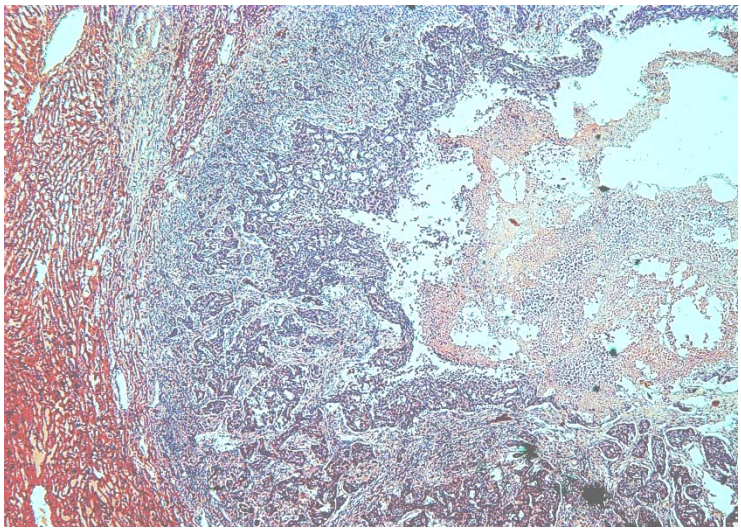


Figure 5. Necrotic fraction and microvessel density of a VX2 tumor at 24 hour follow-up after CKD-516 treatment. Histologic specimen (H&E stain X1) **(a)** and H& E stain X4 **(b)** shows large central necrosis and peripheral viable tumor tissue with necrotic fraction score of 2 (20% to 40%). CD31 staining of the same specimen shows compact tumor cells and sparse microvessels **(c)**.

(a)



(b)



(c)

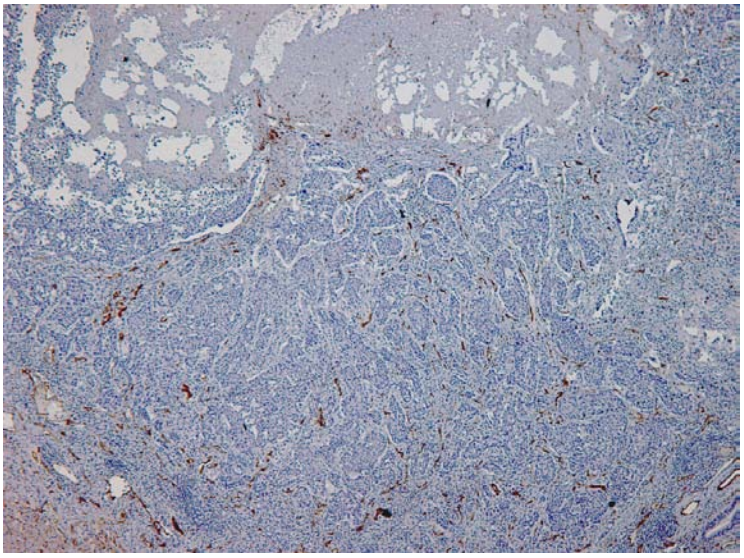
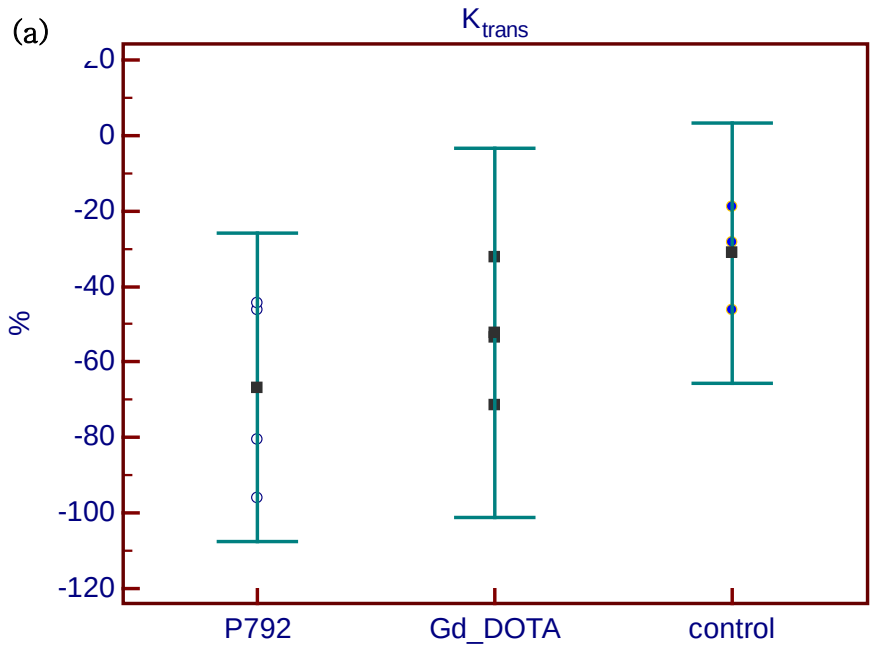
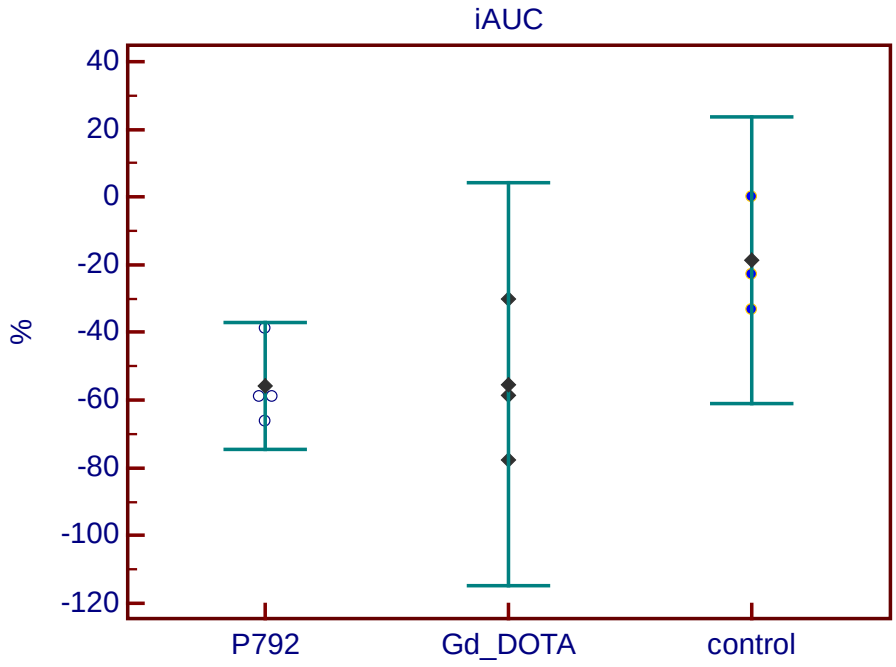


Figure 6. Bar graphs showing relative changes in DCE-MR parameters at follow up studies of the subgroups after Sorafenib treatment. (a) Δ K_{trans} (% x 100) at 14 days, (b) Δ iAUC (% x100) at 14 days after Sorafenib treatment. Error bars indicate 95% CI for mean values.



(b)



DISCUSSION

This study results demonstrated that K_{trans} and iAUC of the liver tumor using macromolecular MR agent, P792-enhanced DCE-MR significantly decreased at 4 hours and 24 hours after VDA (CKD-516) treatment. As a comparison group, Gd-DOTA, which is one of a conventional low molecular MR contrast agents was used, and the DCE-MR result using Gd-DOTA confirmed the previous study results and reproducibility. Furthermore, in our study, by drawing a parallel and comparable conclusion on the antivascular effect using macromolecular MR contrast agent in the DCE-MRI, it was verified that macromolecular MR agent is presumed to be feasible in the DCE-MRI studies of clinical practice.

The result obtained in this study indicate that the macromolecular contrast agent is as effective as the low-molecular weight contrast agent to reveal the treatment effect of antivascular drug, even at doses 4 times lower than the low molecular weight contrast agent. Indeed, K_{trans} and iAUC values of the two contrast agent, P792 and Gd-DOTA estimates were similar in untreated tumors. In all groups, tumors were equally permeable to the

macromolecular contrast agent prior to the CKD-516 or Sorafenib treatment. Owing to the high relaxivity of P792, only quarter of the dose used for Gd-DOTA was sufficient, thereby reducing the possible risk of gadolinium toxicity. This is particularly of concern for patients with preexisting renal disease. Indeed, repeated gadolinium Gd-based extracellular agent administration increases the risk of systemic nephrogenic fibrosis. P792 allows for lower injection dose compared with Gd-DOTA, which may be useful in decreasing the dose of gadolinium.

Furthermore, in our study, the decreasing rate of DCE-MRI parameters after antivascular treatment was overall more prominent in the P792 group compared with Gd-DOTA group. It is generally accepted that low molecular weight gadolinium-based contrast agents often overestimate tumor blood volume and vascular permeability, because of their rapid extravasation from blood to the extracellular space in the tumor tissue. On the other hand, macromolecular contrast agents have been shown to provide more accurate tumor characterization and are better able to differentiate tumor vascular permeability. The significant decrease in tumor microvessel density and therefore endothelial permeability 24 hours

after CKD-516 treatment and 14 days after Sorafenib treatment is consistent with the reduced vessel permeabilities reported in other previous studies where various antiangiogenic drugs and tumor types were used.

When the vascular kinetic parameters are interpreted, the balance between blood flow and capillary permeability in the tissue of interest should be considered. K_{trans} is a function of flow (perfusion) and permeability. In high permeability situations, K_{trans} represents the blood plasma flow, while in cases of low permeability K_{trans} is more influenced by the permeability surface area. After antiangiogenic treatment, tumor vascular permeability is decreased and K_{trans} at this stage more reflects permeability than tumor blood flow. This explains our study result of higher decreasing rate of K_{trans} and iAUC in the P792 group compared with Gd-DOTA group after treatment.

Histologic exam in our study revealed that the degree of tumor necrosis or MVD was not significantly correlated with DCE-MR parameters in both antivascular agent treatment groups. There has been debate regarding the utility of MVD as a biomarker to validate the VDA effect. Histologic MVD may not reflect the functional

vascular properties after VDA treatment. Indeed, K-trans parameter is known to be the most physiologically correlative value with tumor vascularity and permeability, and it is a recommended biomarker for the evaluation of early VDA therapeutic effect.

Sorafenib is an inhibitor of several kinases involved in both tumor cell proliferation (tumor growth) and angiogenesis (tumor blood supply). These include Raf, VEGFR (vascular endothelial growth factor receptor), and PDGFR (platelet derived growth factor receptor). VEGF is the primary mediator of both normal and tumor-associated angiogenesis. It expresses this effect through several mechanisms including induction of endothelial cell division and migration, promotion of endothelial cell survival through protection from apoptosis, and reversal of endothelial cell aging. VEGF interacts with receptors present on the endothelial cell surface. PDGF has its receptor on the surface of capillary endothelial cells. The binding of PDGF to the receptors has several effects on endothelial cell motility and apoptosis.

In the assessment of reproducibility, P792 group showed higher inter-measurement agreement compared with Gd-DOTA group. Overall image quality scoring was also higher in the P792

group, although they did not reach the statistical significance. Considering the higher T1 relaxivity of the macromolecular contrast agent than low molecular agent, this result is anticipatory. Quantitative analysis of contrast enhancement should be accompanied as a further study to elucidate the obvious advantage of macromolecular agent.

CONCLUSION

In conclusion, DCE-MRI using macromolecular MR agent is effective in the demonstration of antivascular drug effect, with lower dose of conventional low molecular MR contrast agent. In addition, macromolecular contrast agent showed that the reproducibility of MR parameter measurement and overall image quality was superior to the conventional low molecular MR contrast agent. Therefore, macromolecular MR agent may be a promising tool in the DCE-MRI for the antivascular treatment monitoring as well as reduced possible risk for gadolinium toxicity.

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국 문 초 록

목적: 고분자 조영제를 이용한 역동적 조영증강 자기공명 영상의 유용성을 토끼 VX2 간암 모델에서의 혈관차단제 치료효과를 평가함으로써 증명해 보고자 하였다.

방법: 27마리의 토끼 VX2 간종양 모델 (CKD-516 치료군 14마리, Sorafenib 치료군 10마리 및 Sorafenib 치료대조군 3마리) 에 대해, 고분자 조영제와 기존의 저분자 조영제를 이용하여 역동적 조영증강 자기공명 영상을 시행하였다. CKD-516 치료군에서는 치료전, 치료 4시간후, 치료 24시간후에 자기공명 영상을 시행하였고 Sorafenib 치료군에서는 치료시작 7일째와 14일째 자기공명 영상을 시행하였다. 자기공명 영상으로부터 얻은 종양의 volume transfer 상수 (K_{trans}) 및 initial area under the gadolinium concentration-time curve until 60 seconds (iAUC) 을 두가지 조영제 군과 각각의 촬영시점에 따라 서로 비교하였다. 또한 이들 자기공명 영상 parameter 들은 종양괴사율과 미세혈관농도를 이용하여 병리소견과의 연관성을 알아보았다. 또한, 자기공명 영상 지표 측정의 재현성과 영상의 질을 평가하여 두 조영제 군 간에 비교하였다.

결과: CKD-516 치료군에서는 고분자 조영제를 이용한 자기공명

영상에서 기존의 저분자 조영제를 이용한 영상보다 치료 4시간 및 치료 24시간째의 K_{trans} 와 iAUC 의 감소 정도가 유의하게 크게 나타났다. Sorafenib 치료군에서는 통계적 유의성은 없었지만, 고분자 조영제 영상에서 치료 7일째와 14일째 K_{trans} 와 iAUC 의 감소 정도가 저분자 조영제 영상과 비교했을 때 더 크게 나타났다. 자기공명영상 지표 측정의 재현성과 전반적인 영상 질은 고분자 조영제를 이용한 영상군에서 좀더 우수하였으나 통계적인 유의성은 나타나지 않았다.

결론: 고분자 조영제를 이용한 역동적 조영증강 자기공명 영상은 혈관차단제의 치료효과 판정 및 추적검사를 하는데 있어서 기존의 저분자 조영제를 이용한 자기공명 영상보다 좀더 유리하고 가돌리늄 부작용의 위험이 적다.

주요어: : 고분자 조영제, 역동적 조영증강 자기공명 영상, 종양혈관 차단제, 간암, VX2 종양

학번: 2007-30502